A New Mechanism of Defence Against Bacteria Through the Use of Certain Foods*

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THE bacteriology of the intestinal tract has been investigated in this laboratory for the last few years and a new viewpoint has been developed (Arnold [1]). Since this review article was published, several contributions have been made which extend our information relative to the adaptation of the bacterial flora within the lumen of the gastrointestinal tract, according to the physiological adaptation of the organism to its environment (Arnold and Kaufman,[2] Nedzel and Arnold,[3] Johnson and Arnold,[4] Nedzel, Stonecipher and Arnold,[5] Seidmon and Arnold,[6] Arnold, Korando and Ryan,[7] and Arnold [8]).

In coöperation with Dr. Walter Eddy of the Teachers College of Columbia University, the use of certain foods has been investigated to determine if they will influence the defensive mechanism of the body against orally ingested bacteria. In order to maintain quantitative relationships we have restricted our experiments to the use of desiccated fruits and vegetables.

The technic outlined by Arnold, Korando and Ryan,[7] who investigated the influence of cornstarch, sucrose, and banana powder on the acid-base equilibrium and flora of the gastrointestinal tract of rats, has been carried out in these studies. This work showed that the use of these three carbohydrate foods did not alter the acid-base equilibrium or the intestinal flora of the gastrointestinal tract. There was, however, a change in the phage susceptibility of the *B. coli* flora. Table I gives the basal diets we have used in these experiments. We have carried out only one small experiment using five rats upon the prune diet. Only a few experiments have been carried out with the tomato diet and three experiments have been completed with the apple diet. The cornstarch and banana diets have been studied for 2 years. This paper will concern itself

TABLE I

Basal Diet

Casein	18 per cent
Butter fat	3 per cent
Cod Liver Oil	2 per cent
Salt Mixture (Osborne & Mendel)	4 per cent
Carbohydrate	68 per cent

Fresh vegetables daily

1 gram dried yeast added daily to feeding

Cornstarch — Control

Banana

* * * *

Apple

Tomato

Prune

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TABLE II

AVERAGE RATIOS OF GRAM-POSITIVE: GRAM-NEGATIVE BACTERIA IN GASTROINTESTINAL SEGMENTS

Diet	*Feces at Beginning of Diet*	*Stomach*	*Duodenum*	*Jej.*$_1$	*Jej.*$_2$	*Jej.*$_3$	*Ileum*	*Caecum*	*Rectum*
Cornstarch	1:3	1:1	4:3	2:1	5:2	3:1	4:1	1:3	1:3
Apple	2:5	2:1	3:1	3:1	5:2	3:1	5:2	2:5	2:3
Banana	1:2	3:1	3:1	5:1	4:1	3:1	5:2	3:5	1:2

mainly with the comparison of cornstarch and banana diets. The information we have upon apple, tomato, and prune is only of a preliminary nature. Table II shows the Gram-positive and Gram-negative ratio of bacteria, determined by direct smears from various segments of the gastrointestinal tract of the rats fed cornstarch, apple, and banana for 3 weeks period of time. Table III shows the hydrogen-ion concentration of the various segments of the gastrointestinal tract, which was carried out on the same segments as reported in Table II. Both of these tables are averages of approximately 300 rats on cornstarch, 300 on banana, and 45 rats on apple diet. It will be seen that both the bacterial flora and the acid-base equilibrium within the lumen of the intestinal tract are approximately the same with the use of these three basal diets.

B. enteritides was fed by stomach tube to rats. The dose was 2.0 c.c. We have used various periods of feeding, but for this paper we will only report upon feeding experiments which were carried out as follows: Rats were placed on the basal diet for 1 week; *B. enteritides* was then administered by stomach tube. The animals were weighed regularly and observed several times each day for diarrhea or other abnormal symptoms. A series of experiments was carried out in which two carbohydrates were used in the diet instead of the single addition as mentioned above. Thirty-four per cent banana powder and 34 per cent cornstarch were used; apple and cornstarch, and tomato and cornstarch were used in the same half and half proportions. Table IV gives the results of a series of such experiments in which diarrhea is recorded in the table. It will be noted that banana, apple, and prune diets protected the rats from diarrhea. Cornstarch and tomato diets did not seem to be protective. When banana was mixed with cornstarch in equal proportions this protective action seemed to have been lost. The tomato mixed with cornstarch did not differ from the tomato diet alone.

The average gain in weight of rats shown in Table IV has been recorded and is shown in Table V. It will be seen that the banana-fed rats have the greatest gain in weight. It does not seem that the growth factor is the one responsible for the resistance enjoyed

TABLE III

AVERAGE HYDROGEN-ION CONCENTRATION OF GASTROINTESTINAL SEGMENTS OF RATS ON VARIOUS DIETS

Diet	*Stomach*	*Duodenum*	*Jej.*$_1$	*Jej.*$_2$	*Jej.*$_3$	*Ileum*	*Caecum*	*Rectum*
Cornstarch	2.71	6.2	6.2	6.12	6.11	6.44	6.36	6.25
Apple	2.75	6.2	6.37	6.22	6.28	6.69	6.73	6.41
Banana	2.88	5.88	6.0	6.18	6.48	6.75	6.58	6.45

TABLE IV

DIARRHEA IN RATS ON VARIOUS DIETS AFTER B. ENTERITIDES FEEDING

Number Animals	*Diet*	*Time of Occurrence of Diarrhea*	*Total Days*
270	Cornstarch	4th to 10th day	6
270	Banana	No diarrhea	0
30	Apple	No diarrhea	0
30	Tomato	5th to 9th, 15th to 19th day	8
5	Prune	No diarrhea	0
15	Banana and Cornstarch	4th to 8th, 14th to 16th day	6
15	Apple and Cornstarch	4th day	1
15	Tomato and Cornstarch	4th to 8th, 14th to 16th day	6

by these animals against diarrhea after enteritides feeding.

Each cage contained 15 rats, in our experiments. Five rats were chosen and labeled, from each cage. Daily cultures of freshly obtained feces were carried out on these 5 rats from each cage. During the first 3 days after *B. enteritides* feeding, the feces were positive in most all instances. After this period of time, one or two colonies were sometimes observed, but there was no correlation between diarrhea and *B. enteritides* in the feces. The cornstarch series of rats showed positive *B. enteritides* longer than any other groups in this series.

Three weeks after the *B. enteritides* was administered, all the animals were sacrificed and organ cultures made from spleen, kidney, liver, heart's blood and the gastrointestinal tract. Considerable variation was found in different cages, even on the same diet, in so far as organ cultures were concerned. Approximately one-third of the animals were found to have *B. enteritides* in the liver; one-fourth were found to have *B. enteritides* in the spleen. The kidney, heart's blood, and the ileum were negative for *B. enteritides*.

We have obtained 21 cultures of the *B. enteritides* group from various laboratories in this country and Europe. A strain sent us by Dr. Karl Meyer of the University of California has proved to be the most virulent strain we have tried in our experiments. This has been designated as the "KM" strain. This strain does not produce regular mortality in rats, even on the cornstarch diet. We have been unable to step up the virulency of this strain by animal passage. Morbidity, based upon the appearance of diarrhea, has been our test of virulency in this study.

B. enteritides isolated from various segments of the gastrointestinal tract and from the feces, as well as from the internal organs of rats fed the cornstarch and the banana diets, have been the same as the original strain, as judged from virulency, biochemical, colony formation, and antigenic tests.

A series of acute experiments were carried out in which rats were killed 1, 3, 6, 24 hours, and 1, 2, and 3 weeks

TABLE V

Diet	*Average Weight at Beginning of Diet*	*Average Final Weight, 4 Weeks on Diet*	*Average Weight Gain per Rat*
	Grams	Grams	Grams
Cornstarch	48	134	86
Banana	48	141	93
Apple	48	100	52
Tomato	60	115	55
Prune	78	114	36
Cornstarch and Banana	67	124	57
Cornstarch and Apple	65	117	52
Cornstarch and Tomato	73	118	45

after feeding *B. enteritides*. This was carried out with rats on cornstarch and banana diets. The distribution of *B. enteritides* within the lumen of the gastrointestinal tract, and in the liver and spleen, was approximately the same in both series.

Graduated doses of *B. enteritides* were injected intraperitoneally in rats to determine the smallest amount causing death in animals. The average dose was found to be 0.25 c.c. of a 24 hour old broth culture. Table VI shows the result of a series of experiments in which we used intraperitoneal injections of the above amount of *B. enteritides*. Three strains were used: the stock " KM " strain, a strain isolated from the feces of banana-fed rats, and a strain isolated from the feces of cornstarch-fed rats. These strains had the same biochemical reactions, colony formation, and antigenic properties. Table VI shows that the banana and cornstarch-fed rats have equal susceptibility to the " KM " strain when injected intraperitoneally. The strains of *B. enteritides* isolated from the feces of the banana-fed rats and cornstarch rats had the same virulency as determined by intraperitoneal injections. In feeding experiments the strains isolated from the banana-fed rats' organs and feces had the same diarrhea producing properties when fed to cornstarch rats as the original " KM " strain. The same was true for *B. enteritides* isolated from feces of cornstarch-fed rats.

TABLE VI

Diet	*Strain B. enteritides*	*Result*
Banana	" KM " stock	Died 48 hours
Banana	Banana feces strains	Died 7–10 days
Banana	Cornstarch feces strains	Died 7–10 days
Cornstarch	" KM " stock	Died 48 hours
Cornstarch	Banana feces strains	Died 7–10 days
Cornstarch	Cornstarch feces strains	Died 7–10 days

Three weeks after *B. enteritides* intragastric administration to rats on banana diet, intraperitoneal injection of the minimal lethal does of the same antigen caused death on the 3rd and 4th day. The same experiment carried out with cornstarch-fed, enteritides-injected rats produced death on the 7th and 8th day. This would indicate some protection in the cornstarch-fed rats as a result of their experience with this antigen. These results are not conclusive but may indicate some protection.

Heat stable Salmonella toxins and ox-bile (Arnold [1]) were given by mouth to the banana-fed rats in order to increase their susceptibility to *B. enteritides*. These gastrointestinal irritants did not seem to disturb the animals inasmuch as no diarrhea developed and the organ cultures were the same as the control series.

TABLE VII

FIFTY MICE ON EACH DIET FOR 1 WEEK, THEN FED B. ENTERITIDES AND OBSERVED FOR 4 WEEKS

Diet	*Per Cent Mortality*
General stock	96.0
Cornstarch	42.0
Banana	6.0

The first of the series of experiments upon mice will be included in this paper. Our stock diet for mice consists of equal amounts of " puppy meal " and cracked corn. This has been used for a number of years in this institution for our stock mouse colony. Table VII gives the results of the use of 150 mice, 50 for each of the three diets indicated. After 1 week on these respective diets, the animals were given *B. enteritides* by stomach tube and observed for 4 weeks. The mortality of those on the stock diet was 96 per cent, cornstarch diet 42 per cent, and the banana diet 6 per cent. These experiments are being continued. This table is included to indicate that mice may be better animals for assay of these factors than rats.

DISCUSSION

Standard basal diets, with variations in the carbohydrate components, were fed from the standpoint of calories, protein, inorganic salts, and vitamins. The diet promoted growth, produced good pelts, and the behavior of the animals was normal as far as we could determine. The differences in the results obtained, after the *B. enteritides* feeding, was first thought to be due to an interference with the self-disinfecting mechanism (Arnold [1]). But, inasmuch as the intra-intestinal distribution was the same in both series of animals, this did not explain the absence of diarrhea in the banana-fed animals. We next thought of decreased permeability of the intestinal wall as a factor. But, inasmuch as the organ cultures were approximately the same in both series of animals, this mechanism again did not explain the differences in these experiments. When banana powder is used for the carbohydrate source of our basal diet instead of cornstarch, rats have the power of physiologically adapting themselves to *B. enteritides* administered by mouth. The intra-enteric and organ distribution of *B. enteritides* is the same in both banana- and cornstarch-fed series. No resistance is enjoyed by the banana-fed rats 3 to 6 weeks after *B. enteritides* injection, although one-third to one-fourth of these animals are liver and spleen carriers of *B. enteritides*. Wasserman and Citron [10] explain the increased protection enjoyed after local vaccination as due to a re-tuning of the endothelial system and a consequent increase in physiological response by destroying invading bacteria *in situ*. Inasmuch as the bacteria in the organs are the same in both series, this explanation would not hold in our experiments.

Gulbrandsen [9] discussed before the Laboratory Section of this meeting, a change of bacteria from viable to non-viable forms within the stomach and duodenum. He showed that these same non-viable forms could be rendered viable again under certain conditions. In other words, the active acid-secreting stomach has more of a bacteriostatic than a bactericidal function. There is a complicated biological change going on within the lumen of the intestinal tract. Bacteria are rendered non-viable within the acid-secreting stomach and are revived and made viable in the lower segment of the small intestine and in the large intestine. In our experiments so far, when these non-viable forms penetrate the wall of the intestinal tract and are cultured from the internal organs, they show certain biochemical and antigenic alterations from the original strain. We are now investigating this aspect of the protective action of foods against pathogenic enteric bacteria, in laboratory animals.

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